CATACHEM

CREATINE KINASE (CK)
MANUAL/AUTOMATED PROCEDURE
C184-0A, C184-0B, V184-12

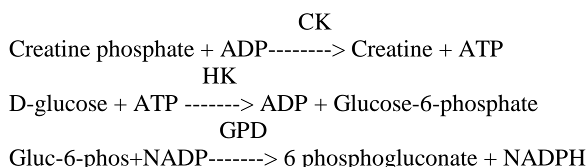
Intended Use
For **IN VITRO quantitative** determination of Creatine Kinase (Creatine Phosphokinase) in serum.

Clinical Significance
Measurements of Creatine Kinase activity are primarily used for diagnosing skeletal muscle disease, myocardial infarction, cerebrovascular accidents, muscular dystrophy, hypothyroidism, pulmonary infarctions, as well as for monitoring the causes and treatments. (1)

Method History
In 1955 Oliver (1) described a method for Creatine Kinase (EC 2.7.3.2) determination using creatine phosphate and adenosine diphosphate as substrate. Oliver's method was jointly optimized by the Scandinavian Committee on Enzymes and the German Society for Clinical Chemistry (2, 3, 4). In this procedure N-acetylcysteine (NAC) is the thiol activator and adenosine monophosphate (AMP) and p1-p5-di (adenosine) pentaphosphate (AP5A) are added to inhibit the interference caused by the adenylate kinase activity. The Catachem Creatine Kinase procedure is based upon the recommendations of the Scandinavian Committee on enzymes and the German Society for Clinical Chemistry.

Method Principle
The Creatine Kinase enzyme catalyzes the conversion of creatine phosphate and adenosine diphosphate to creatine and ATP. The resultant ATP is quantitatively determined by coupling a hexokinase (HK) reaction and a glucose-6-phosphate (GPD) reaction to the CK reaction.

GPDH catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconic acid with concomitant reduction of NADP. The amount of NADPH produced is proportional to the CK activity present in the serum sample and its concentration is measured by the increase in absorbance at 340nm. The reaction scheme illustrates the reactions that occur in this method.



Reagent Content
When reconstituted according to the directions, the concentrations of the active ingredients in the reagents will be approximately as follows:

Creatine Kinase Reagent
Each liter contains:

Creatine Phosphate	35.0 mmol
ADP	2.0 mmol
NADP	2.0 mmol
NAC	20.0 mmol
AMP	5.0 mmol
AP5A	10.0 umol
D-Glucose	20.0 mmol
Hexokinase	≥3000 U
GPD	≥2000 U

Nonreactive ingredients and stabilizer

Creatine Kinase Diluent
Each liter contains:
Buffer
Nonreactive ingredients and stabilizer

Precautions
Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagents
Prepare the required number of vials of Creatine Kinase Working Reagent by adding the corresponding Diluent volume to the CK Reagent. Mix well for 2-3 minutes.

Reagent Storage And Stability
Store the Creatine Kinase Reagents at 2-8˚C. When stored as directed, these reagents are stable until the expiration date stated on the label. Store the Creatine Kinase Working Reagent (liquid) at 2-8˚C. When prepared and stored as directed, the Creatine Kinase Working Reagent is stable for at least seven days.

Specimen Collection And Preparation
Test sera should be fresh, clear and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In separated unhemolyzed serum the CK enzyme concentration is stable for eight hours at 25˚C and 72 hours at 2-8˚C. (5)

Quality Control
To monitor the performance of the Working Reagents and the procedure used, we recommend the regular use of normal and abnormal control serum.

Interfering Substances
Several substances have been reported to alter the CK activity in serum (6, 7). A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (8)

Expected Values (9)

Adult Human Males:	24 - 195 U/L (37˚C)
Adult Human Females:	24 - 170 U/L (37˚C)

These values are suggested guidelines. Values are species dependent. Each laboratory should establish its own normal range for the species under test and for the area from which the samples are taken.

Procedure: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.05 ml for sample

Graduated cylinder 25 ml or 50 ml for reagent

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Materials Provided

Catachem Creatine Kinase Reagent and Creatine Kinase Diluent

Analytical Parameters

Wavelength	340 nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	Rate
Reaction Time	5 minutes
Reagent Volume	1.0 ml
Sample Volume	0.05 ml
Total Volume	1.05 ml
Sample-to-reagent ratio	1:21

Assay Procedure

1. Set spectrophotometer wavelength at 340nm and zero the instrument with the cuvette containing water.
2. Pipette 1.0 ml of Working Reagent into each of two cuvettes marked “Sample” and “Control”.
3. Incubate cuvettes for 2.0 minutes at 37˚C.
4. Pipette 0.05 ml of Control or Sample into their respective cuvettes. Mix all cuvettes well.
5. Replace the cuvettes in spectrophotometer and continuously monitor the change in absorbance for at least 5 minutes.
6. Read the “Control” and “Sample” absorbencies.
7. Calculate the CK concentration (U/L) in the sample(s), as shown in calculations and results.

Results And Calculations

One international unit (U/L) is defined as the amount of enzyme that catalyzes the conversion of one micromole of substrate per minute under the defined conditions:

$$CK\ (U/L) = \frac{\Delta\ OD/min \times 1.05\ ml \times 1000}{6.22 \times L\ (cm) \times 0.05\ ml}$$

Where:

Delta A/Min	=	change in abs/min
1.05 ml	=	total reaction volume in ml
1000	=	converts U/ml to U/L
6.22	=	extinction coefficient
L (cm)	=	1 cm reaction cuvette
0.05 ml	=	of sample used in assay

Example: Δ OD/Min = 0.025

$$CK\ (U/L) = \frac{0.025 \times 1.05\ ml \times 1000}{6.22 \times 1\ cm \times 0.05\ ml} = 84.4\ U/L$$

Method Performance Characteristics

Sensitivity: 0.00023-0.00028 absorbance units/U/L
Linear Range: 0-1200 U/L
Precision: Within-run and day-to-day precision is summarized below:

Precision Study

CK	TOTAL		WITHIN-RUN	
MEAN	SD	CV	SD	CV
U/L	U/L	%	U/L	%
110	2.60	2.40	1.70	1.50
571	12.70	2.20	5.70	1.00
1014	23.00	2.30	7.80	0.70

Correlation

A comparison of this method using an automated analyzer and a reference method based upon the recommendations of the Scandinavian Committee on Enzymes resulted in the following regression statistics.

Range:	=	24 - 2107 U/L
N:	=	120
Y:	=	0.985x + 2.4
r:	=	0.999
Sy.x:	=	8.1

References

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6. Martin EW. Hazards of Medication (Alexander SF, Farage DJ and Hassan WE Jr. eds) Philadelphia, PA and Toronto, Canada. JB Lippincott Co (1971) pp1699.
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